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Prediction of Successful Outcome in a Randomised Controlled Trial of the Long-Term Efficacy of Interferon Alpha Treatment for Chronic Hepatitis C

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To evaluate the efficacy of a 12-month course of recombinant interferon alpha (IFN-α2b), and to assess predictive factors of successful response to IFN therapy in chronic active hepatitis C (HCV CAH), 242 patients with histologically proven HCV CAH were assigned randomly to two groups, one treated with IFN-α2b (3 MU three times weekly, intramuscularly), the other untreated. To determine the efficacy of IFN-α2b 12 months after therapy, a second liver biopsy was carried out on 100 treated patients and 27 untreated patients. The biochemical, virological, and serological response of patients followed up for at least 50 months after treatment was also evaluated to confirm the efficacy of IFN-α2b. The genotypes of infecting HCV, anti-HCV core IgM, and HCV-RNA concentrations were also analysed and the predictors of response determined by univariate and multivariate analyses. Response was defined in terms of the normalisation of aminotransferase activities and the disappearance of HCV-RNA. The overall long-term response was 39.4%. Anti-HCV core IgM levels were significantly lower in long-term responders. Patients with increased levels of IgM anti HCV core (>3.8 sample/cut-off), infected with genotype 1b were nonresponders. Liver histology improved significantly in patients with long-term response. Multivariate analysis identified three independent predictors of the likelihood of long-term response to IFN therapy: age younger than 40 years, basal anti-HCV core IgM levels ≤ 3.8, and genotypes other than 1b. These data indicate that the treatment with IFN-α2b used in this randomised controlled trial is effective in HCV CAH. Anti-HCV core IgM was the strongest predictor of long-term response in the present study. ***J. Med. Virol. 58:26–34, 1999.***

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INTRODUCTION

Chronic active hepatitis C virus (HCV CAH) is a common and often progressive viral liver disease in at least 40–50% of patients with acute hepatitis C [Koretz et al., 1985; Dienstag and Alter, 1986]. Although patients may remain asymptomatic over extended periods and experience only marginal elevations of aminotransferase, cirrhosis appears to develop in a significant percentage of those affected [Dienstag and Alter, 1986] and many of those affected are at increased risk of developing hepatocellular carcinomas. The two aims of hepatitis C treatment are the early elimination of replicating HCV from liver and blood, and the resolution of biochemical and histological signs of chronic hepatic inflammation. Several preliminary reports [Hoofnagle et al., 1986; Davis et al., 1989; Di Bisceglie et al., 1989] have suggested that 50–70% of patients with HCV CAH may respond to a standard 3-month course of interferon-α (IFN-α), although about half will relapse once treatment has been stopped. Recent trials

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have suggested that a 12-month course may be more effective in nonresponders [Poynard et al., 1996].

Criteria for the identification of responders and nonresponders are still the object of intense study. Many retrospective analyses have identified several host and viral factors associated with an increased probability of sustained long-term response to therapy with IFN-α [Camps et al., 1993; Tsubota et al., 1994; Conjeevaram et al., 1995; Yamada et al., 1995]. Early age, recent occurrence of the disease, low HCV-RNA levels, absence of cirrhosis, low hepatic iron, and low genetic complexity of infecting HCV quasi-species [Kanazawa et al., 1994; Enomoto et al., 1995] prior to treatment are dominant factors. In addition, a few studies have indicated that increased basal levels of anti-HCV core IgM in patients with chronic hepatitis C are a predictor of poor response to IFN-α [Tassopoulos et al., 1994; Vandelli et al., 1994; Pawlotsky et al., 1995]. Conversely, patients with low or nondetectable levels of anti-HCV core IgM may be more likely to respond to IFN-α treatment [Tassopoulos et al., 1994]. This study set out (1) to explore, in a randomised, controlled study, the long-term efficacy of a 12-month course of 3 MU IFN-α2b for the treatment of chronic hepatitis C; (2) to identify pretreatment variables predicting a long-term sustained response to this treatment schedule; and (3) to evaluate the role of anti-HCV core IgM in monitoring patients during therapy with IFN-α treatment.

PATIENTS AND METHODS

Patients

Between 1990 and 1991, 242 consecutive patients (150 men, 92 women, mean age 46.5 ± 9.5 years) diagnosed with chronic hepatitis C were enrolled in our Liver Unit, in a randomised, controlled trial of a 12-month course of IFN-α2b (Table I). Recombinant IFN α-2b was given at a dose of 3 MU three times weekly (tiw), intramuscularly (IM) for 12 months. A total of 121 patients received IFN-α2b (treatment group) and 121 remained untreated (control group). Patients assigned to the control group did not receive placebo. All patients had persistent serum aminotransferase elevations, on aggregate more than twice the upper normal limit for at least 6 months prior to enrolment in the study. Liver biopsy abnormalities were consistent with chronic persistent hepatitis (CPH) in 1 patient, CAH in 223 (155 had mild and 68 severe CAH), and cirrhosis in 18. Patients with decompensated liver disease, other serious medical conditions, or evidence of other forms of liver disease were excluded from the study. Serum hepatitis B surface antigen was negative and iron, transferrin, and copper levels were normal in all cases. Patients were monitored at weekly intervals during the first month and monthly thereafter. Blood samples were taken for blood counts and liver function tests, including measurements of serum alanine (ALT) and aspartate (AST) aminotransferases, total bilirubin, albumin and total protein. After the treatment period, all treated patients (except 4 who dropped out) were followed up for 50 months, whereas 27 untreated patients were followed up until the second liver biopsy.

TABLE I. Baseline Clinical, Biochemical, Histological, and Virological Features in Patients With Chronic Hepatitis C

Characteristics	Treated group	Control group
No. of cases	121	121
Age (years)[a]	45 ± 10	46 ± 9
Male/Female	86/35	64/57
Source of HCV infection		
Intravenous drug addiction	2	0
Blood transfusion	12	9
Analytical data		
Aspartate aminotransferase (U/L)[a]	94 ± 38	89 ± 21
Alanine aminotransferase (U/L)[a]	155 ± 48	138 ± 48
Bilirubin (mg/dl)[a]	0.78 ± 0.25	0.18 ± 0.27
Albumin (g/dl)[a]	4.01 ± 0.35	4.03 ± 0.4
HBsAg+	0	0
Anti-HBs+	2	5
Anti-HBc+	2	5
Anti HCV+	121	121
RIBA III	121	121
HCV-RNA (by PCR)+	112	115
HCV-RNA (by bDNA)+	104	ND
HCV-RNA level (MEq/ml)	1.8 ± 2.3	ND
HCV genotype		
1b	77	79
2a	26	27
3	8	9
Mixed[b]	1	0
Anti-HCV core IgM (S/CO)	4.4 ± 1.3	4.1 ± 0.9
Histology		
CPH	1	0
CAH mild	74	81
CAH severe	37	31
Cirrhosis	9	9

CPH, chronic persistent hepatitis; CAH, chronic active hepatitis; ND, not determined; S/CO, sample/cut-off; PCR, polymerase chain reaction; bDNA, branched DNA; HCV, hepatitis C virus.
[a]Data are presented as means ± SD. Differences between the two groups are not statistically significant (P = NS, ANOVA test).
[b]Mixed infection with genotype 1 and 3.

The study protocol was approved by the local Ethics Committee and written informed consent was obtained from all the subjects admitted to the trial.

Viral Markers

Commercially available radioimmunoassays were used to determine hepatitis B surface antigen and antibodies to surface and core antigens (AUSRIA, AUSAB, CORAB; Abbott Laboratories, North Chicago, IL). Antibodies against HCV were determined by second-generation enzyme-linked immunosorbent assay (ELISA) and confirmed by RIBA II (Ortho Diagnostic, Raritan, NJ) supplementary assay.

Detection, Quantification, and Typing of Serum HCV-RNA

Serum HCV-RNA was detected by the polymerase chain reaction using two sets of primers specific for the 5′ untranslated region (5′-UTR) of HCV genome in a nested amplification format [Tisminetzky et al., 1994].

HCV genotyping. HCV genotypes were identified by reverse transcription-polymerase chain reaction (RT-PCR) of the 5′UTR as described previously [Tisminetzky et al., 1994]. Briefly, cDNA was synthesised from 3 μl of patient sera and then amplified by nested PCR using a set of universal primers targeting the 5′UTR (AS1:-GTTCCACGGTCTACGAGACCT-3′, S1: 5′-GCCATGGCGTTAGTATGAGT-3′, S2:-5′-GTGCAGCCTCCAGGGGGACCC-3′, and AS2: 5′-CCGTGAGCGTTCGTGGGATA-3′). Amplification products were spotted in triplicate on nylon membrane and hybridised with the following ^{32}P-labelled probes (HCV-1: 5′-CGCTAATGCCTGGAGAT-3′, HCV-2: 5′-CACTCTATGCCCGGCCAT-3′, and HCV-3: 5′-CGCTCAATACCCAGAAAT-3′). PCR products were digested with BstUI enzyme to distinguish subtype 1a from subtype 1b.

Serum HCV-RNA was further quantified using a branched DNA assay (bDNA assay, Quantiplex HCV-RNA 2.0 assay, Chiron Corporation, Emeryville, CA) according to the manufacturer's instructions. Specimens with HCV-RNA levels exceeding the cut-off value of the kits (i.e., 0.2 HCV-RNA MEq/ml) were considered positive. All assays were carried out in duplicate, and the mean quantitation value of the duplicates was calculated. The final result was reported in HCV-RNA MEq/ml.

Anti-HCV Core IgM

An anti-HCV Core IgM EIA Kit (Abbott GmbH, Diagnostika, Wiesbaden) was used to detect anti-HCV core IgM antibodies, according to the manufacturer's instructions. Briefly, 10 μl of serum prediluted in specimen diluent were incubated at 40°C for 150 min with a 0.25-inch plastic bead coated with full-length recombinant core protein. Unbound materials were removed by washing, and captured IgM antibodies were detected by incubating the bead-antigen-antibody complex for 60 min at 40°C with a solution containing horseradish peroxidase-labelled goat antibodies directed against human class IgM antibodies (μ-chain specific). Beads were then washed and a solution containing *o*-phenylene diamine and H_2O_2 was added. After a 30-min incubation at room temperature in the dark, the colour reaction was stopped by adding 1 N H_2SO_4, and the resulting change in colour was measured as optical density at 492 nm (OD 492). All assays for IgM anti HCV core were run in duplicate. HCV core IgM antibody levels were expressed as mean specimen to cut-off (S/CO) optical density (OD) ratio in samples at baseline and, respectively, at months 1, 6, 12 during treatment, as well as 6 and 12 months after therapy.

Liver Histology

Liver biopsy was taken at baseline with consent from all patients. After 12 months of treatment-free follow-up, a second liver biopsy was taken from 100 treated and 27 untreated patients. Coded sections stained with haematoxylin-eosin, Perls and Gomory stains were evaluated blind for comparative purposes by two observers, who were unaware of the clinical data. Liver biopsies were classified morphologically and the degree of necroinflammatory activity (grade) and fibrosis (stage) scored, following Ishak et al. [1995].

Other Measurements

Serum autoantibodies were investigated by indirect immunofluorescence technique. Serum rheumatoid factor (RF) was sought in all patients using a nephelometrec analysis. Human interferon alpha (Hu-IFN-α) was quantitated at baseline using a sandwich immunoassay, Human IFN-α, Elisa Kit, (Biosource International, Camarillo, CA).

Definition of Response

Complete response (CR) to treatment was defined as the normalisation of serum ALT levels, and the disappearance of HCV-RNA at the end of treatment. Patients whose ALT aminotransferase activities decreased by more than 50% during treatment, but failed to return to normal, were considered partial responders (PR). Patients with no change in ALT were considered nonresponders (NR). Any patients exhibiting ALT elevations and reappearance of HCV-RNA during follow-up after the treatment period were classified as relapsers (CR/rel). Patients with normal ALT and HCV-RNA negativity, at the end and 50 months after therapy withdrawal, were considered long-term sustained responders (LTR).

Statistical Analysis

Results are presented as means ± SD and/or ± SE. Data were evaluated by ANOVA, paired and unpaired Student's *t*-test, and correlation coefficient regression analysis. Univariate analysis was undertaken to identify factors associated with long-term response to interferon therapy. For odds ratio (O.R.) estimates, the reference category was that with the lower proportion of long-term responders (age > 40 years, female sex, ALT > 100 UI/L, anti HCV core IgM > 3.8, genotype 1b, and bDNA > 2.8). Stepwise logistic regression analysis was used to identify independent predictors of sustained response.

RESULTS

Of the 242 patients admitted to the study, 223 (92.2%) were affected by HCV-associated chronic active hepatitis, 18 (7.4%) had cirrhosis, and 1 (0.4%) had CPH. At baseline there were no significant differences between patients assigned to treated/untreated groups as to age, sex, source of HCV infection (where known), biochemical, virological, and histological features. Table I shows the clinical, histological, and virological features of the study population. Two patients dropped out before completing the study. Most patients in the trial had community-acquired infection (88.4%), 12 (9.9%) treated and 9 (7.4%) untreated patients had previously received a blood transfusion, and 2 (1.6%) had a history of intravenous drug abuse. Serum autoantibodies were positive for antinuclear antibodies (ANA) < 1:40 in 3 treated patients and in 2 patients in the con-

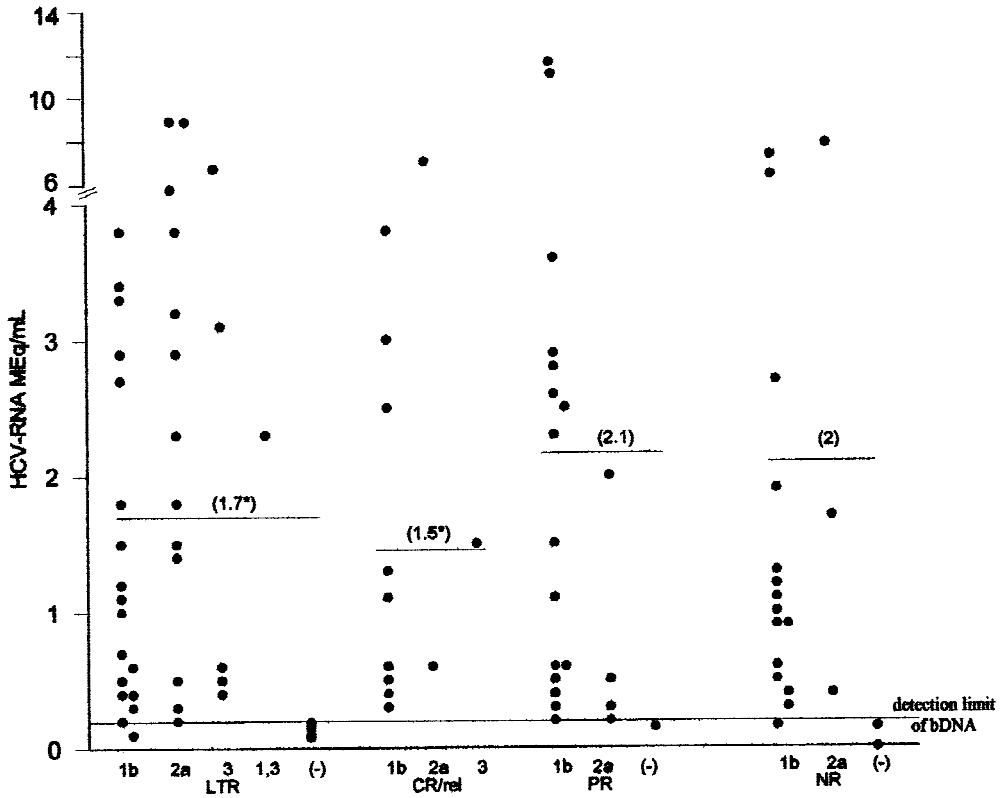

Fig. 1. Serum hepatitis C virus (HCV) RNA levels as a function of HCV genotype and therapy outcome. LTR, long-term responders; CR/rel, responders with relapse; PR, partial responders; NR, nonresponders; (–), HCV-RNA negative by polymerase chain reaction; 1b, 2a, 3, and 1,3, HCV genotypes. Horizontal bars denote mean value of serum HCV-RNA levels. HCV-RNA concentration, estimated by branched DNA probe assay (Chiron), is given as MEq/ml. *$P < .05$ compared with PR and NR.

trol group; rheumatoid factor was negative in all patients. Two treated and 5 untreated patients were anti-HBc and anti-HBs positive.

A significant reduction in serum ALT and AST levels was observed only in the treatment group; 87.2% of the patients who showed a response to the IFN-α therapy did so within the first 3 months of treatment. Aminotransferase levels completely normalised during, or by the end of the 12-month treatment period in 68 treated patients, i.e., a CR of 57.1%, but in only 1 untreated patient. In complete responders, ALT levels remained in the normal range during the treatment-free follow-up period in all but 19 patients, i.e., CR/rel of 27.9%, in whom biochemical reactivation of disease occurred, respectively, 3, 6, 9, and 12 months after therapy. Forty-seven of the 68 CRs (69.1%, 2 patients in this group were lost to follow-up) showed a sustained response during the 50-month, treatment-free follow-up, hence an overall LTR of 39.4%. In 25 patients, ALT levels decreased by at least 50% without, however, achieving normalisation during treatment, i.e., a PR of 21%. Twenty-six patients were nonresponders, i.e., a NR of 21.8%.

Serum HCV-RNA at entry was positive by PCR in 112 treated patients (92.6%), becoming undetectable with a beneficial response to treatment, in 46 patients (38.9%). At entry, 104 (86%) of the treated patients were detected positive by bDNA 2.0. Figure 1 shows individual patients' serum HCV-RNA levels (MEq/ml), according to the infecting genotype, on the basis of response to IFN. Mean serum HCV-RNA levels were lower in patients with response to IFN therapy (LTR and CR/rel) than those in patients with a PR (2.1 MEq/ml) or NR (2.0 MEq/ml). However, the difference was not statistically significant.

Prior to treatment, 77 patients (63.6%) were found to be infected with HCV genotype 1b, whereas 26 (21.5%) were infected with genotype 2a, 8 (6.6%) with 3, and 1 (0.8%) with mixed genotype 1 and 3. The remaining 9 cases (7.4%) could not be genotyped because of a negative HCV-RNA result at the time of pretreatment liver biopsy. These 9 patients with increased serum ALT level were HCV-RNA positive in serum samples tested 6 months and 1 month earlier as outpatients. Response rates according to genotypes are shown in Table II. Patients infected with HCV genotypes 2a and 3 showed increased long-term sustained response than those infected with genotype 1b ($P < .05$). At baseline, anti-HCV core IgM tested positive by HCV IgM EIA 2.0 in 86 patients (71.5%). Anti-HCV core IgM levels were significantly lower in LTR (1.8 ± 0.6) than those in PR (5.8 ± 1.8) and NR (6.4 ± 1.6) ($P < .001$). Patients relapsing after a complete response had intermediate anti-HCV core IgM levels (3.5 ± 1.3). Figure 2 displays individual patients' HCV core IgM antibody levels, according to infecting genotype, on the basis of response

TABLE II. Distribution of the HCV Genotype According to Response to IFN

HCV genotype	Dropped-out (n = 4)	LTR (n = 47)	CR/rel (n = 19)	PR (n = 25)	NR (n = 26)
1b	2 (2.6)	19 (24.7)	16 (27.2)	19 (24.7)	21 (20.8)
2a	1 (3.8)	15 (57.7)	2 (7.7)	5 (19.3)	3 (11.5)
3	1 (12.5)	6 (75)	1 (12.5)	—	—
Mixed[a]	—	1 (100)	—	—	—
NEG	—	6 (66.6)	—	1 (11.1)	2 (22.3)

n = number of cases. Numbers in parentheses are percentages.
HCV, hepatitis C virus; IFN, interferon; LTR, long-term responders; CR/rel, responders with relapse; PR, partial responders; NR, nonresponders; NEG, negative.
[a]Mixed infection with genotype 1 and 3.

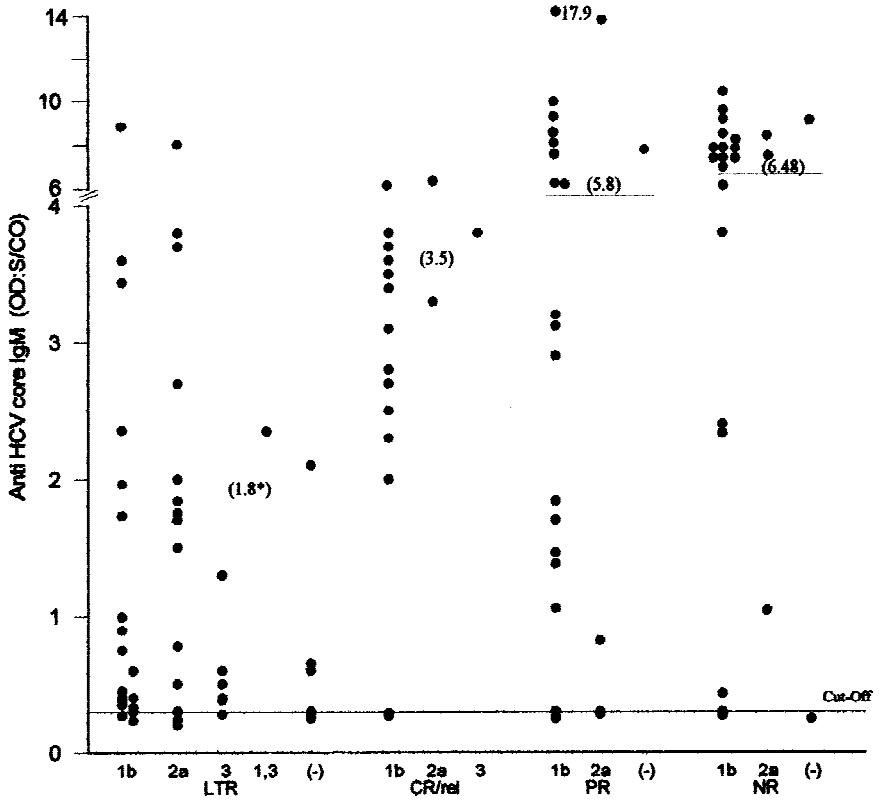

Fig. 2. IgM anti-hepatitis C virus (HCV) core levels at the start of interferon-α (IFN-α) treatment as a function of HCV infecting genotype and therapy outcome. LTR, long-term responders; CR/rel, responders with relapse; PR, partial responders; NR, nonresponders; (–), HCV-RNA negative by polymerase chain reaction; 1b, 2a, 3, and 1,3, HCV genotypes; OD:S/CO, optical density:sample cut-off. Horizontal bars denote mean value of IgM anti-HCV core serum levels. *P < .001 compared with PR and NR.

to IFN-α2b. Most patients who relapsed or did not respond to IFN therapy were infected by HCV type 1b and had increased anti-HCV core IgM levels. Conversely, patients with lower anti-HCV core IgM levels were more likely to achieve a long-term sustained response, despite being infected by genotype 1b. Nonetheless, long-term sustained response in patients infected by genotype 1b was poor, as confirmed by a relapse rate of 78.9% (15/19).

Mean anti-HCV core IgM levels computed for each response group at the start of therapy, at months 1, 6, and 12 during therapy, and at 6 and 12 months after therapy are summarised in Table III. LTR and CR/rel showed a significant reduction in their mean HCV core IgM levels during the first half of therapy (months 1–6, P < .001), which was not observed in NR or PR patients. Long-term sustained responders maintained low or undetectable anti-HCV core IgM. After an initial significant decline observed during the first 6 months of treatment, relapsers maintained low but detectable anti-HCV core IgM (≤ 2.0) during the treatment-free follow-up period. On the other hand, partial responders significantly reduced their anti-HCV core IgM during the second half of therapy to intermediate levels (2.0–4.0, S/CO) (P < .001), which they maintained after treatment. Nonresponders tended to react similarly during the second half of the treatment period; however, their anti-HCV core IgM levels were not con-

TABLE III. Summary of anti-HCV core IgM Values (S/CO) Before, During, and After IFN-α Treatment in Patients With Chronic Hepatitis C

Months[a]	LTR	CR/rel	PR	NR
0	1.8 ± 0.6*	3.5 ± 1.3	5.8 ± 1.8	6.4 ± 1.6
1	1.6 ± 0.5	2.2 ± 0.8	5.6 ± 1.8	6.6 ± 1.9
6	1.2 ± 0.4**	1.5 ± 0.6**	5.2 ± 1.7	5.8 ± 1.3
12	1.2 ± 0.4	2.1 ± 0.9	4.4 ± 1.6**	4.8 ± 1.1
18	1.1 ± 0.4	1.6 ± 0.5	4.4 ± 1.7	6.1 ± 1.6
24	1.1 ± 0.7	1.6 ± 0.3	4.5 ± 1.3	6.2 ± 1.2

Data are expressed as means ± SE.
HCV, hepatitis C virus; IFN-α, interferon-alpha; S/CO, sample/cut-off; LTR, long-term responders; CR/rel, responders with relapse; PR, partial responders; NR, nonresponders.
[a]Months: 0 = therapy starting point; (1, 6, 12) = successive times of therapy; (18, 24) = subsequent moments of follow-up.
*$P < .001$ (LTR vs. NR).
**$P < .001$ compared with pretreatment values.

trolled and increased again to reach their high pretreatment levels (i.e., > 4.0, S/CO). IgM anti-HCV core and HCV-RNA positivity concurred in 71.5% of sera. Nevertheless, anti-HCV core IgM levels did not correlate with HCV-RNA levels as measured by bDNA ($r^2 = 0.0297$, P = NS) and similar results were obtained in LTR ($r^2 = 0.0423$, P = NS, data not shown). There was no significant difference in anti-HCV core IgM positivity between HCV genotypes: 81.3% in 1b, 72.0% in 2a, and 85.7% in type 3. No correlation was found between IgM anti-HCV core levels and the characteristics of the viral strain or histological score of liver disease (P = NS). Although anti-HCV core IgM levels tended to be higher in patients infected by HCV type 1b (S/CO 4.3 ± 1.2, mean ± SE) than in patients infected by other genotypes, the difference was not statistically significant. Conversely, low anti-HCV core IgM titres were not associated with HCV type 2a and 3. However, the small number of patients infected by HCV genotype 2a and 3 in this study prevented us from drawing any final conclusions.

Liver biopsy specimens were available from all patients before therapy. After 12 months of treatment-free follow-up, a second liver biopsy was available in 100 (82.6%) treated and in 27 (22.3%) untreated patients. There were no significant differences in liver histology between the two groups before therapy (Table I). Comparison of paired liver biopsy specimens revealed a significant improvement in all patients achieving normal ALT levels. In long-term sustained responders, posttreatment liver biopsy showed normal liver histology in 9 cases (19.1%): 2 diagnosed with severe and 7 with moderate CAH, and minimal nonspecific changes in 22 cases (46.8%) (Fig. 3). Patients classified as LTR and CR/rel displayed a significantly higher rate of histological improvement ($P < .001$) compared with partial or nonresponders. The most prominent histological improvement observed in patients treated with IFN-α2b was in the degree of current hepatic injury; in particular, piecemeal hepatocyte necrosis and lobular injury benefited from the treatment. Interestingly, liver histology was also found to have improved in approximately 30% of both partial and nonresponders. In the control group, liver histology deteriorated in 20 of 27 patients (74%), whereas in 7 patients (25.9%), no significant changes were observed.

Prior to treatment, neither HCV-RNA, nor anti-HCV core IgM were found to be associated with the histological picture of the liver. However, the absence of a sufficient number of cases with minimal histological activity ($n = 0$) or with CPH ($n = 1$) and the relatively low number of patients with cirrhosis ($n = 18$) admitted to the trial precluded a proper statistical assessment.

Possible factors that contributed to the efficacy of IFN-α2b therapy first underwent univariate analyses, and those that reached statistical significance ($P < .05$) were then entered into a multiple logistic stepwise regression model for multivariate analysis. Initial variables included age, sex, baseline ALT, histological activity, HCV genotype, basal HCV-RNA, anti-HCV core IgM levels, and human interferon levels. Univariate analysis indicated that an age of less than 40 years, anti-HCV core IgM levels ≤ 3.8, and genotype other than 1b were associated with a long-term response, whereas gender, basal ALT levels, and bDNA were not. Multiple logistic regression analysis identified age ≤ 40 years, anti-HCV core IgM ≤ 3.8, and genotypes other than 1b as independent predictors of the likelihood of long-term response to interferon therapy. The strongest association was found with anti-HCV core IgM ≤ 3.8 (O.R. 8.6; CI 95% = 1.8–42.4) (Table IV).

Side Effects

In the early stages of IFN therapy, flu-like symptoms (fever, asthenia, and myalgia) were common and self-limiting. In two cases, weight and hair losses were observed, but symptoms returned to normal when treatment was stopped. None of the patients withdrew from treatment because of drug-related side effects.

DISCUSSION

Pilot studies [Hoofnagle et al., 1986] and several randomised, controlled trials have demonstrated the efficacy of IFN-α for chronic hepatitis C treatment [Davis et al., 1989; Di Bisceglie et al., 1989; Marcellin et al., 1991]. Only 20–25% of patients treated with a regimen of 3 MU, three times a week, for 6 months [Saracco et al., 1993; Castillo et al., 1994] achieve a sustained biochemical and virological response. In this study, the overall sustained response rate (LTR) was 39.4% with a follow up of 50 months. This rate contrasts with some studies [Davis et al., 1989; Di Bisceglie et al., 1989; Marcellin et al., 1991], but is consistent with the observation of other authors [Iino et al., 1993; Mazzella et al., 1994; Negro et al., 1994; Chemello et al., 1995]. Longer courses of therapy may be more beneficial, offering a better-sustained response rate than a shorter 6-month course [Saracco et al., 1993; Reichard et al., 1994; Poynard et al., 1995]. Furthermore, recent meta-analyses indicate that a 12-month therapy generates a significantly higher response rate [Poynard et al., 1996]. The variability of results could be explained by

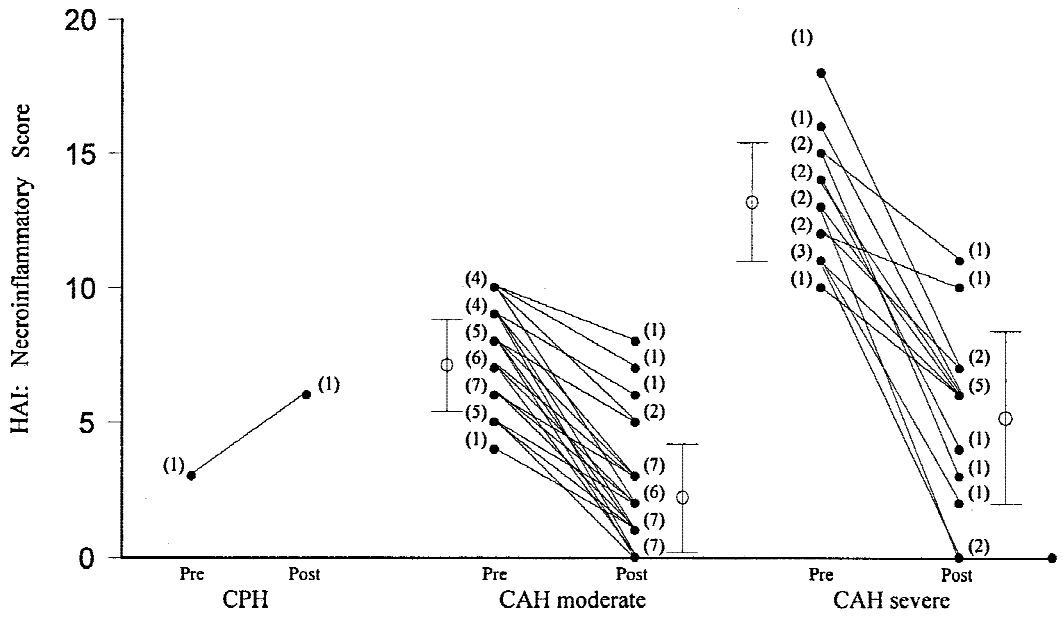

Fig. 3. Changes in ranking of hepatic injury on histologic examination in long-term responders before and after receiving interferon. CPH, chronic, persistent hepatitis; CAH, chronic, active hepatitis; HAI, histological activity index of grading according to Ishak et al. [1995]. Injury (portal inflammation, piecemeal necrosis, and lobular injury) was measured as described in Methods. The open circles indicate mean ± SD values at each time in each group. Numbers in parentheses indicate number of cases.

TABLE IV. Factors Associated With the Likelihood of Long-Term Response to Interferon Therapy

Variable	No. LTR/No. treated	(%)	O.R. (CI 95%) crude	O.R. (CI 95%) adjusted
Age (years)				
> 40	30/92	(32.6)	1	1
≤40	17/25	(68.0)	4.4 (1.6–12.7)	3.7 (1.2–11.4)
Sex				
Females	11/34	(32.4)	1	
Males	35/82	(42.7)	1.4 (0.4–1.7)	
ALT (U/L)				
>100	32/85	(37.6)	1	
≤100	15/32	(46.9)	1.5 (0.6–3.6)	
Anti HCV core IgM (S/CO)				
>3.8	2/28	(7.1)	1	1
≤3.8	45/89	(50.6)	13.3 (2.8–nd)	8.6 (1.8–42.4)
Genotype				
1b	19/75	(25.3)	1	1
Others	22/33	(66.7)	5.9 (2.2–16.0)	5.8 (2.1–16.0)
bDNA (MEq/ml)				
≤2.8	35/97	(36.1)	1	
>2.8	12/20	(60.0)	2.7 (0.9–8.0)	

No., number of cases; LTR, long-term responders; O.R., odds ratio; CI, confidence interval.
Crude and adjusted odds ratios (O.R.) were derived by multiple logistic regression analysis.

differences in clinical and virological features (prevalence of cirrhosis, HCV type, viral load). A further explanation could be that, in this study, there were young people and a small number of cirrhotic patients. Indeed, several studies have emphasised that the absence of cirrhosis and young age are associated with a better response to IFN therapy [Camps et al., 1993; Tsubota et al., 1994]. On the other hand, the low number of drug addicts, the absence of homosexual patients, and the low incidence of blood transfusions could have influenced the rate of response.

After a year's treatment, the patients in the treated group showed a significant reduction in viral replication, and 38.6% of them became HCV-RNA negative. These data are in line with those reported by other authors [Iino et al., 1993]. The histological features improved in 46.8% of LTRs with a decrease in the histological activity index. Moreover, histological lesions could no longer be detected in 19.1% of LTRs. The absence of viremia at the end of treatment and changes in liver histology appear to be the most important factors in monitoring a long-term response. Furthermore, the

follow-up period after therapy withdrawal should last at least 1 year because a late reactivation of the disease might be observed. The simultaneous presence of IgM anti-HCV core and HCV-RNA in 71.5% of patients in the present study suggests that the presence of IgM anti-HCV core is basically related to viral replication, although an IgM negative test does not exclude viremia. Nevertheless, IgM anti-HCV core did not correlate with the characteristics of the viral strains or with the concentrations of circulating virus quantified by bDNA. Perhaps this finding is not surprising. IgM antibody detection partly measures an immune response to HCV, and this response is likely to vary in different patients with similar levels of viremia. It has been suggested that the presence in the serum of IgM anti-HCV core antigen correlates with the activity of liver disease [Papatheodoridis et al., 1997] and has a predictive value for a subsequent response to IFN-α therapy [Brillanti et al., 1992; Tassopoulos et al., 1994; Yuki et al., 1995; Tabone et al., 1997]. In this study, IgM anti-HCV core did not correlate with the histological score of liver disease. Similarly, Quiroga et al. [1995] did not find any relationship between IgM anti-HCV core and disease severity or progression. Pawlotsky et al. [1995] found a prevalence of anti-HCV core IgM significantly higher in patients infected with genotype 1b than in patients with other genotypes. Although the mean levels of IgM anti-HCV core antigen, were higher in patients infected by HCV type 1b than in patients infected by other genotypes, no significant difference was observed in our data. The levels of IgM anti-HCV core antigen were significantly lower in long-term responders than in partial or nonresponders. The relapse rate could not be predicted by basal anti-HCV core IgM levels or by the behaviour of its levels during treatment, as anti-HCV core IgM levels decreased significantly after 6 months of IFN therapy in all responders. Many studies have shown that some HCV genotypes and the lack of heterogeneity in the E2/NS1 region of the HCV genome have been associated with an improved response to IFN [Okada et al., 1992; Tsubota et al., 1994]. Patients infected with genotype 1b, according to Simmonds' classification [Simmonds et al., 1993], are less likely to respond to IFN therapy [Mahaney et al., 1994; Kanai et al., 1995]. In the present study, patients with a low level of IgM anti-HCV core responded well to IFN therapy, regardless of the infecting genotype. Conversely, infected people with high levels of IgM anti-HCV core displayed a lack of response to IFN. Other authors [Mahaney et al., 1994; Chemello et al., 1995; Kanai et al., 1995] have obtained similar results in patients infected by genotype 1b, by increasing either the dose of IFN or the duration of treatment.

Age younger than 40 years, low anti-HCV core IgM level, and genotype other than 1b are independent predictors of favourable response to IFN therapy. Patients infected with genotype 1b appear more likely to relapse than those infected with other genotypes, particularly when associated with high IgM anti-HCV core levels. Whether relapse is related to high HCV mutation rates and/or to a selection of viral subpopulation resistant to IFN [Enomoto et al., 1994; Pawlotsky et al., 1994] is not yet clear. Other authors have found cirrhosis to be a major factor determining absence of response; in the present study, however, liver histology was not found to influence the response to IFN therapy. The small proportion of patients with cirrhosis in our study, and the fact that there was a similar level of basal histological activity in the treated patients, might explain this discrepancy.

In conclusion, the results of this study confirm that IFN-α2b is clinically effective in patients with chronic hepatitis C who have been treated according to this schedule, and the findings suggests that the follow-up period after therapy withdrawal should last at least 1 year to ascertain a long-term response. IgM anti-HCV core test turned out to be the strongest predictor of IFN response in this study. Monitoring patients' anti HCV core IgM levels prior to and during treatment might be of use in designing alternative therapeutic strategies in those patients infected with HCV genotype 1b.

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